Available online at www.sciencedirect.com

ScienceDirect

South African Journal of Botany 76 (2010) 453–459

SOUTH AFRICAN JOURNAL OF BOTANY

www.elsevier.com/locate/sajb

# Seed germination at different temperatures and water stress levels, and seedling emergence from different depths of *Ziziphus lotus*

M. Maraghni[1], M. Gorai*[,1], M. Neffati

*Laboratoire d'Ecologie Pastorale, Institut des Régions Arides, Médenine 4119, Tunisia*

Received 14 September 2009; received in revised form 17 February 2010; accepted 26 February 2010

**Abstract**

*Ziziphus lotus* (L.) Lam. is a deciduous shrub with intricately branched stems in the Rhamnaceae family. It's a dominant and economically important species widely distributed in active sand dunes in the southern desert of Tunisia. To provide basic information for its conservation and reintroduction, we studied the influence of environmental factors on seed germination patterns. The germination responses of seeds were determined over a wide range of constant temperatures (10–50 °C), polyethylene glycol (PEG)-6000 solutions of different osmotic potentials (0 to −1 MPa) and burial depths (1–10 cm). Temperatures between 15 and 45 °C seem to be favorable for the germination of this species. Germination was inhibited by either an increase or decrease in temperature from the most suitable temperature found (35 °C). The highest germination percentages (100%) were obtained under control conditions without PEG, and increasing moisture stress progressively inhibited seed germination, which was less than 5% at −1 MPa. When tested for germination in distilled water, after PEG treatments, seeds germinated to the same extent as when fresh. When seeds buried deeply, there was a significant decrease in seedling emergence percentage and rate. Seedlings of *Z. lotus* emerged well at depths of 1–2 cm and could not emerge when sand burial depth was >4 cm.


*Keywords:* Burial depth; Osmotic potential; Seed germination; Seedling emergence; Temperature; *Ziziphus lotus*

## 1. Introduction

Tunisia is one of the countries most seriously affected by desertification. Accounting for three quarters of the country (Le Houérou, 1959), southern Tunisia subdivides in two zones: (i) the first zone known as arid covers $5.5 \times 10^4$ km$^2$ with average annual precipitations between 100 and 350 mm and (ii) the second zone known as desert with an annual average rainfall lower than 100 mm occupies an area of about $6.5 \times 10^4$ km$^2$ (PNUD/FAO, 1979). One of the promising options for restoration of decertified regions in southern Tunisia is to use native shrub and tree species that have multiple functions in the ecosystem. Several native species are potentially useful for dune stabilization and extension of plant cover, including some *Ziziphus* species.

The genus *Ziziphus* belongs to the family Rhamnaceae, represented by 135–170 species (Bhansali, 1975; Liu and Cheng, 1994) with evergreen or deciduous trees or shrubs usually armed with unequal stipular spines. Of these, only *Z. spina-christi* (L.) Willd, *Z. vulgaris* Lam. and *Z. lotus* (L.) Lam. are found in Tunisia (Laamouri and Zine El Abidine, 2000). Although similar, *Z. lotus* differs from the other two species by its deciduous shrubby habit with intricately branched stems and smaller flowers and fruits (Jafri, 1977). This species is indigenous to Tunisia and known as "Sedra" has a wide ecological and geographical distribution in both climatic regions of Tunisia and grows under a variety of environmental conditions. It is a shrub that reaches 2–5 m and is found in depressions with deep sandy soil. Mounds composed of wind-borne sediment that accumulated around *Z. lotus* thorn scrub have long been reported from the Tunisian steppe regions (Tengberg and Chen, 1998). This species is a dominant perennial shrub in active sand dunes and stabilized sand fields in the southern arid zone of Tunisia. *Ziziphus lotus* is dormant from October through March and mature plants flower in May and June and produce fruits in August (Gorai et al., unpubl. data). The edible fruit called a nabk is a subglobose dark yellow drupe (c. 1–1.5 cm in diameter) at maturity and have dark-brown seeds (c. 6 × 5 mm). The leaves are valuable animal forage and

* Corresponding author. Tel.: +216 75633005; fax: +216 75633006.
*E-mail address:* gorai.mustapha@yahoo.fr (M. Gorai).
[1] Authors have participated equally to this work.

0254-6299/$ - see front matter 
doi:10.1016/j.sajb.2010.02.092

fodder under open grazing conditions. Recently, the anti-inflammatory, analgesic and anti-spasmodic activities of this plant were demonstrated in rodents (Borgi et al., 2008; Borgi and Chouchane, 2009). These characteristics make *Z. lotus* a valuable multi-purpose shrub for semi-arid to arid ecological areas.

*Ziziphus* seeds are enclosed within a hard woody endocarp known as the stone which is sometimes wrongly referred to as the seed. Establishment of *Ziziphus* species is constrained by poor germination and seedling emergence, mainly as a result of a stony endocarp, and dormancy types. Baskin and Baskin (1998, 2004) have proposed a comprehensive classification system which includes five categories of seed dormancy: physiological (PD), morphological (MD), morphophysiological (MPD), physical (PY) and combinational (PY + PD). Analyzing the seed phylogenetic tree based on the internal morphology of the embryo and endosperm in mature angiosperm seeds, Finch-Savage and Leubner-Metzger (2006) signalled that seeds of Rhamnaceae family presented non dormancy (ND), PD, PY, and PY + PD. Baskin and Baskin (1998) reported that physical and physiological dormancies are about equally important among desert shrubs, but a few species including *Ziziphus* ssp. have nondormant seeds. Abrasion of the endocarp happens naturally over time from the action of fire, wind and water, microbial attack and exposure to high and fluctuating temperatures. For controlled germination, physical dormancy is broken in seeds of *Ziziphus* species by two main categories: mechanical scarification, and non-mechanical methods (Pareek, 2001). It is now evident that both methods enhanced emergence of *Ziziphus* species as compared to those without removal endocarp. Stone passage through the gut of vertebrates is critical for endozoochorus seed dispersal because it enhanced germinability for some *Ziziphus* species. A large proportion of seeds excreted by spider monkeys and capuchins for *Z. cinnamomum* (Zhang and Wang, 1995), by cattle for *Z. mauritiana* (Grice, 1996) and by foxes for *Z. mistol* (Varela and Bucher, 2006) contain viable seeds that can germinate.

Seed germination of *Ziziphus* species is affected by the initial percentage viability at the time of seed collection, and by storage conditions, environmental conditions at sowing time, and treatments applied to break dormancy (Pareek, 2001; Azam-Ali et al., 2006). As indicated by Pareek (2001), the storage at reduced temperatures of $4.5 \pm 0.5$ °C in perforated polythene bags result in retention of viability for longer periods. Applying three different cold stratification durations (20, 40 and 60 days) at $5 \pm 1$ °C, Olmez et al. (2007) observed that the highest germination percentage was 14.1% for *Z. jujuba* seeds without removal of the endocarp when stratified for 20 days and sown under greenhouse conditions. Studying the germination rate on *Z. mauritiana*, Grice (1996) found that seeds collected from the soil surface declined from a rate of 56% in the control (fresh dehulled seeds) to 31 and 20% after 6 and 12 months, respectively. The same author indicated that germination rate of seeds buried at depth of 2 cm declined to 7% after 6 months. For the same species, Srimathi et al. (2002) found that cv. Umran seeds from fruits collected from the crown exhibited higher germination rates than those collected from the ground. The degree of fruit ripeness may affect germination of the enclosed seed. In Senegal, Danthu et al. (1993) observed that seed germination of *Z. mauritiana* increased from 2% for seeds from green fruits to 28% when fruits are more mature and have turned yellow and 56% for seeds from fully ripe fruits that have turned red; however the rate declines to 46% for seeds from overripe fruits that have turned brown.

The establishment of plants in arid regions is often limited by temperature when moisture conditions are favourable (Evans and Etherington, 1990). Knowledge of temperature effects on germination may be useful to evaluate the germination characteristics or the establishment potential among range species (Jordan and Haferkamp, 1989). In the field, sand temperature near the surface in the daytime can be considerably higher than air temperatures (Zhang and Maun, 1990); however, when the sand near the surface is moist, the only condition in which seeds germinate, evaporative cooling will prevent an extreme rise in sand temperature. Temperature changes may affect a number of processes controlling seed germinability, including membrane permeability and the activity of membrane-bound and cytosolic enzymes (Bewley and Black, 1994). Another crucial factor determining germination and seedling growth is soil moisture and, therefore, plays an important role in determining the distribution patterns of species (Gutterman, 1993). Both seed germination rate and final germination percentage decrease with reduced soil water potential (Tobe et al., 2006; Daws et al., 2008; Gorai et al., 2009). Besides temperature and moisture, burial depth plays an important role in seed germination for plants living in sandy desert where seeds are often deeply buried or exposed to air due to sand movement (Tobe et al., 2006). Germination of seeds was directly related to the seed size and the depth at which seeds were buried (Bond et al., 1999; Ren et al., 2002). Burial at shallow depths stimulated more germination than surface lying seeds because it maintains a moist environment around seeds and prevented seeds and seedlings from drying out (Huang and Gutterman, 1998). However, excessive burial may affect seed germination and prevent the seedling emergence above the sand. Comparing 10 *Calligonum* species, Ren et al. (2002) found that percentage of germination decreased quickly as the burial depth increased. The risk of desiccation-induced mortality may be lower for seedlings from large seeds since such seedlings can emerge from greater soil depths (Bond et al., 1999) and have more rapid radicle growth rates (Daws et al., 2007) than seedlings from small seeds. Large seed size may also facilitate germination and establishment in large gaps by enabling emergence from greater soil depths (Bond et al., 1999) where soil drying will be less rapid.

There has been little experimental research done on *Z. lotus* and we attempt to investigate the factors controlling seed germination and seedling emergence of this plant species, as well as to determine seed dormancy type. Information from this study provides basic knowledge about germination and emergence that can be used for re-establishing projects.

## 2. Materials and methods

### 2.1. Seed collection

Fruits of *Z. lotus* were obtained from wild plants which were collected from a location near Ben Guerdane (33°17′N, 10°55′E; Southeast Tunisia) in September 2007. This area is arid to semi-arid with a typical Mediterranean climate, characterized by irregular rainfall events and a harsh dry summer period. Annual precipitation is around 186 mm and annual mean temperature is 19.4 °C with a minimum temperature 3.9 °C in January and 35.9 °C maximum in August correspond to climatic data where original population of the plants occurred. Fruits were cleaned and stored for six months in the seed bank of the Laboratoire d'Ecologie Pastorale at the Institut des Régions Arides (Médenine, Tunisia) in which relative humidity was set at 30% and temperature was maintained at 20 °C. When experiments were carried out, fruit pulps were removed and endocarps were cracked using a manual peeler. The stones contain two seeds embedded in the endocarp. Individual seeds of *Z. lotus* weigh 33 ± 3.8 mg (mean ± SE, $n$ = 20, Maraghni and Gorai, unpubl. data).

### 2.2. Effects of constant temperatures on seed germination

Seeds were surface sterilized in 0.58% sodium hypochlorite for 1 min, subsequently washed with deionized water and air-dried before being used in experiments to avoid fungus attack. To determine the most suitable temperature for germination, seeds were sown on two layers of filter paper (Whatman No. 1) in a 90-mm glass Petri dish with 5 ml of deionized water and incubated at 10, 15, 20, 25, 30, 35, 40, 45 and 50 °C in darkness (Lumінincube II, analys, Belgium; MLR-350, Sanyo, Japan). A completely randomised design was used in the germination tests. For each treatment, four replicates of 25 seeds each were used. During 20 days the number of germinated seeds were counted and removed every 2 days. A seed was considered to have germinated when the emerging radicle elongated to 2 mm. Three characteristics of seed germination were determined: final germination percentage, number of days to first germination (delay of germination) and mean time to germination (MTG). MTG was estimated according to the formula: $MTG = \Sigma (n_i \times d_i)/N$, where $n_i$ is the number of germinated seeds at day $i$, $d$ the incubation period in days and $N$ the total number of germinated seeds in the treatment.

### 2.3. Effects of osmotic potential on seed germination and recovery

The effects of osmotic potential on seed germination were examined by incubating seeds, as described in the first experiment, at the most suitable temperature found with PEG-6000 solutions of known osmotic potential ($\Psi_\pi$): 0, −0.2, −0.4, −0.6, −0.8 and −1.0 MPa (Michel and Kaufmann, 1973). PEG-6000 solutions were renewed every 48 h under sterile conditions to ensure relatively constant $\Psi_\pi$ in the treatments. All seeds from the previous germination tests which did not germinate after 20 days at different PEG-6000 solutions, were placed in new Petri dishes with filter paper moistened with deionized water, and incubated under the same conditions for additional 20 days to study the recovery of germination. Recovery, for each specific osmotic environment, was calculated as the fraction of ungerminated seeds after PEG treatment that germinated in the subsequent germination test in deionized water.

### 2.4. Effects of burial depth on seedling emergence

The effect of burial depth on seedling emergence was studied in a semi-field experiment. Seed sowing was performed in April 2008 and climatic data are shown in Table 1. All sand burial treatments consisted of four replicates of 25 seeds per pot. For each replicate, seeds were sowed at the depth of 1, 2, 4, 6, 8 and 10 cm in plastic pots (20 cm in diameter) filled with sandy soil. A completely randomised design was used in the sand burial depth tests. The pots were perforated at the bottom and containing a layer of gravel to ensure drainage of water. Pots were watered daily with rainwater through the experimental period. During 30 days the emerged seedlings (cotyledons visible at the sand surface) were counted daily and removed. At the end of the experiment, the contents of pots were removed and washed over a sieve with 2 mm openings through which the sand escaped but the ungerminated seeds and unemerged seedlings (seeds germinated but the seedlings etiolated below sand surface) were recorded. Because this experiment focused mainly on seedling emergence, all dormant or decomposed seeds were recorded as non-germinated. Three characteristics of seedling emergence were determined: final emergence percentage, number of days to first emergence (delay of emergence) and mean time to-emergence (MTE). MTE was estimated according to the formula: $MTE = \Sigma (n_i \times d_i)/N$, where $n_i$ is the number of emerged seedlings at day $i$, $d$ the incubation period in days and $N$ the total number of emerged seedlings in the treatment.

### 2.5. Statistical analysis

Germination and emergence percentages were arcsine transformed before statistical analysis to ensure homogeneity of variance. Data were analysed using SPSS for windows, version 11.5. A one-way analysis of variance (ANOVA) was performed on all results. Tukey test (Honestly significant differences, HSD) was used to estimate least significant range between means.

## 3. Results

### 3.1. Effects of temperature and osmotic potential on seed germination

In response to the tested constant temperatures, most germination (100%) of *Z. lotus* occurred at 35 °C and none at

Table 1
Mean climatic data during the month of April in semi-field for burial depth experiment of *Ziziphus lotus* seeds.

| PP (mm) | T (°C) | TM (°C) | Tm (°C) | H (%) | V (km $h^{-1}$) |
|---|---|---|---|---|---|
| 2.79 | 22.2 | 27.5 | 14.2 | 38.6 | 12.4 |

H: mean humidity, PP: precipitation amount, T: mean temperature, TM: maximum temperature, Tm: minimum temperature and V: mean wind speed.

10 or 50 °C (Fig. 1). Temperature significantly affected the final percentages of germination (Table 2). During the first four days, most germination, and fastest germination, occurred at 35 °C. Seed germination was highest in deionized water control (100%) and germination percentages declined with a decrease in $\Psi_\pi$ (Fig. 2), less than 5% of the seeds germinated at −1 MPa. With a reduction of $\Psi_\pi$, the number of days to first germination was delayed. The MTG was significantly affected by temperature and PEG-6000 treatment based on the results of one-way ANOVA (Table 2). Germination rate increased with increasing temperature from 15 to 35 °C (Fig. 1) and decreased with decreasing water potential from 0 to −1.0 MPa (Fig. 2). After 20 days of PEG-6000 treatments, seeds were transferred to deionized water to determine the recovery of germination. A one-way ANOVA indicated that recovery was significantly affected by water stress levels (Table 2). The presented results show that ungerminated seeds from PEG-6000 treatments recovered when stress conditions were alleviated (Table 3).

### 3.2. Seedling emergence from seeds sown at different depths

At the end of the 30-day experiment, seeds germinated at all burial depths from 1 to 10 cm. In contrast, seedling emergence was highly influenced by burial depth increase (Fig. 3; Tables 3 and 4). The highest seedling emergence occurred from 1 cm burial depth. There was a significant decline in seedling emergence at 4 cm burial depth, and none emerged from depth reached 6 cm. The fastest seedling emergence occurred from 1 cm burial depth and there was a significant increase in days of first emergence at 2 and 4 cm burial depths (Table 4). Logarithmic regression analysis was used to determine the relationships between germinated seeds that did not emerge and emerged seedlings at different burial depths. There were strong relationships between seed germination and seedling emergence according to the burial depth with $R^2=0.84$ and 0.89, respectively (Fig. 3).

Table 2
Results of a one-way ANOVA of seed germination and seedling emergence characteristics of *Ziziphus lotus* by temperature, PEG-6000 solutions and burial depth.

| Factors | Dependent variables | | |
|---|---|---|---|
| | Germination percentage (%) | MTG (days) | Germination recovery (%) |
| Temperature | 111.88 | 39.32 | – |
| PEG-6000 | 63.11 | 96.23 | 14.60 |
| | Emergence percentage (%) | MTE (days) | First day of emergence (days) |
| Burial depth | 15.89 | 27.96 | 173.59 |

Data represent *F*-values significant at $P<0.001$.

## 4. Discussion

It is evident that germination and emergence of *Ziziphus* seeds are inhibited by a stony endocarp. Unfortunately, similar to several other tropical and subtropical shrubs, the seeds exhibit mechanical scarification to overcome physical dormancy. In the present study, the impermeable endocarps of *Z. lotus* fruits were artificially broken to release seeds that can achieve 100% germination at 35 °C (Fig. 1). By comparing seeds from soaked and un-soaked endocarps of *Z. lotus* in water, it appears that they could be described as having a form of mechanical dormancy imposed by the physical restriction from the endocarp. As a result, seeds from soaked endocarps have taken up water compared to un-soaked seeds that have been removed from endocarps. Several other studies revealing that removal of the endocarp can achieve higher percentages of germination and emergence on jujube species than those of intact stones such as *Z. mauritiana* (Prins and Maghembe, 1994; Grice, 1996), *Z. nummularia* (Hussain et al., 1993), *Z. abyssinica* (Prins and Maghembe, 1994), *Z. mucronata*

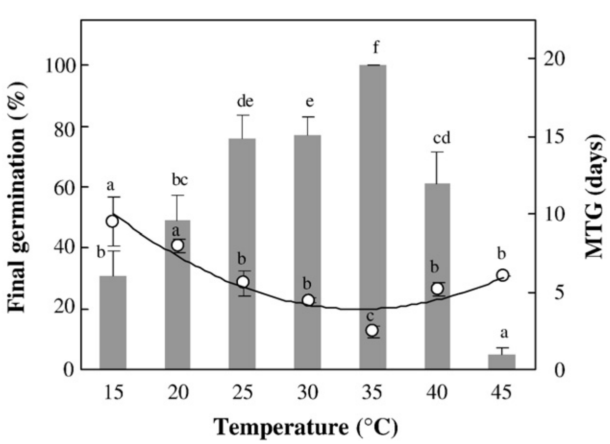


Fig. 1. Variation of the final germination percentages (%) and the mean time to germination (MTG, days) of *Ziziphus lotus* seeds during 20 days at different temperatures (15–45 °C). A line describing the evolution of MTG was obtained by polynomial regression. At 10 and 50 °C seed germination was completely inhibited. Values of each parameter (mean ± 95% confidence limits, $n=4$), having the same letter are not significant different ($P>0.05$) from each other (Tukey test).

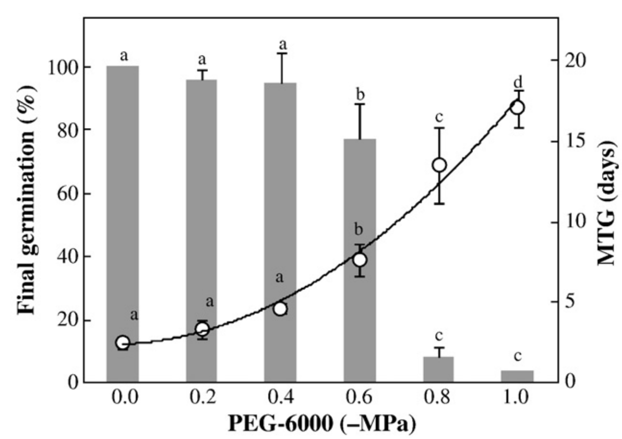


Fig. 2. Variation of the final germination percentages (%) and the mean time to germination (MTG, days) of *Ziziphus lotus* seeds during 20 days at different PEG-6000 solutions of different osmotic potentials (0 to −1 MPa). A line describing the evolution of MTG was obtained by polynomial regression. Values of each parameter (mean ± 95% confidence limits, $n=4$), having the same letter are not significant different ($P>0.05$) from each other (Tukey test).

Table 3
Mean germination (%) at 35 °C of *Ziziphus lotus* seeds indicating the recovery after 20 days of transfer to deionized water and total germination (mean ± 95% confidence limits, $n = 4$).

| PEG-6000 (−MPa) | Germination (%) | |
|---|---|---|
| | Recovery | Total |
| 0.6 | 69 ± 24.09 | 92 ± 5.5 |
| 0.8 | 79 ± 13.5 | 81 ± 12.5 |
| 1.0 | 71 ± 7.4 | 72 ± 7.1 |

(Hassen et al., 2005; Griffiths and Lawes, 2006), *Z. spina-christi* (Saied et al., 2008) and *Z. joazeiro* (Alves et al., 2008). According to Baskin and Baskin (1998), temperature requirements for shrubs in hot semi-deserts and deserts to achieve 60–100% germination range from 15 to 35 °C, with temperatures of about 20–25 °C being suitable for most species. In the case of the studied species, these percentages were reached from 25 to 40 °C. However, At 10 and 50 °C germination was completely inhibited (Fig. 1). Studying 3 jujube species including *Z. lotus*, Laamouri and Zine El Abidine (2000) found high percentages of germination at 25 °C. This variation in the most suitable temperature found in the former study and our experiments may be controlled by the maternal parent and environmental effects. Temperature requirement for germination have been determined for a number of jujube species. Speer and Wright (1981) observed that average temperatures for germination of *Z. obtusifolia* var. *obtusifolia* seeds in the laboratory were 20 to 30 °C. According to Aráoz et al. (2004) for *Z. mistol* and Laamouri and Zine El Abidine (2000) for *Z. lotus*, *Z. vulgaris* and *Z. spina-christi* germination is preferred at 25 °C. For *Z. mauritiana*, higher percentages of germination were observed from 30 to 35 °C (Danthu et al., 1993). However, others studies were conducted at alternating temperature regime of 30/20 °C and 28/20 °C for *Z. mucronata* (Hassen et al., 2005; Griffiths and Lawes, 2006, respectively) and 30/25 °C for *Z. spina-christi* (Saied et al., 2008).

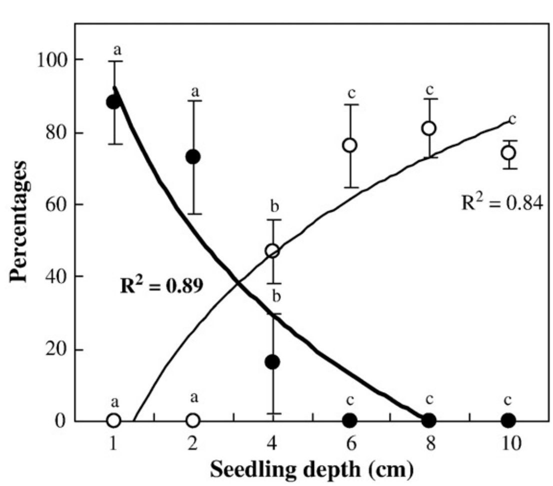


Fig. 3. Regression plots for mean germinated seeds that did not emerge (○) and emerged seedlings (●) of *Ziziphus lotus* at different burial depths (1–10 cm). Lines describing the course of these two parameters were obtained by logarithmic regression. Values of each parameter (mean ± 95% confidence limits, $n = 4$), having the same letter are not significant different ($P > 0.05$) from each other (Tukey test).

Table 4
Number of days to first emergence seedling and mean time to emergence (MTE) of *Ziziphus lotus* seeds at various depths for 30 days. At burial depth > 4 cm, no emergence was recorded.

| Depth (cm) | Germination characteristics | |
|---|---|---|
| | First day of emergence (days) | MTE (days) |
| 1 | 11 ± 0.57 a | 13.89 ± 0.49 a |
| 2 | 14 ± 1.88 b | 18.65 ± 1.64 b |
| 4 | 21 ± 2.47 c | 24.08 ± 2.78 c |

Means within a column followed by the same letter are not significantly different at the $P = 0.05$ level for the given burial depths (mean ± 95% confidence limits, $n = 4$).

*Ziziphus lotus* is one of the most common Rhamnaceae found in many regions in arid Tunisia producing large fruit numbers that come to maturity in August. Our data indicate that seeds were viable over a wide range of constant temperatures from 15 to 45 °C in darkness (Fig. 1). Seeds of many shrubs germinate equally well in light and darkness, and those of some species germinate to higher percentages in darkness than in light (Baskin and Baskin, 1998). In our experiments, it should be noted, however, that seeds were removed from dark incubators every 2 days to check germination; thus, seeds may have received enough light to promote germination. In the former case it is difficult to conclude on Light: dark requirement of *Z. lotus.* Hussain et al. (1993) found that seeds of *Z. nummularia* germinate when covered by soil, indicating that they can achieve germination in darkness. Although some seeds of *Z. obtusifolia* var. *obtusifolia* germinated without light and without a cold treatment, both of these factors tripled germination when they were present (Speer and Wright, 1981). Others studies emphasized the importance of light for germination of *Z. mistol* (Del Longo and Aráoz, 2009), *Z. mucronata* (Hassen et al., 2005; Griffiths and Lawes, 2006) and *Z. spina-christi* (Saied et al., 2008).

In saline and dry soils, water potential is not very different to that of desiccated seeds. Therefore, at low osmotic potentials water does not enter the seeds and induces germination. Desert shrubs vary in their ability to germinate in the presence of moisture stress. In our experiments, it should be noted that moisture stress was tested at the most suitable temperature found (35 °C). According to Scifres and Brock (1969), seeds of *Prosopis juliflora* are more tolerant of moisture stress at an optimum germination temperature (29 °C) than they are at temperatures above and below it. Seeds of *Z. lotus* germinated to 95 and less than 5% in PEG-6000 solutions of −0.4 and −1 MPa (Fig. 2). By increasing moisture stress, similar results were found on germination of *Diospyros texana* seeds that decreased from about 95 to 45% at 0 and −0.6 MPa, respectively (Everitt, 1984), whereas germination of three deciduous semi-shrubs of genus *Artemisia* was inhibited severely in PEG-6000 solutions at −1.2 MPa (Tobe et al., 2006). Comparing 14 neotropical pioneer species, Daws et al. (2008) found that germination occurred both more quickly and at lower water potentials with increasing seed mass. These authors suggested that the positive association of seed size and canopy gap size for optimal seedling establishment is maintained by differential germination responses to soil water availability coupled with the scaling of radicle growth rate and seed size, which collectively confer greater drought tolerance on large-seeded species. Seed imbibition rate, germination percentage and germination rate generally decrease as soil water potential decreases (Song et al., 2005), either by drought or by higher salinity. Seed-soil contact has been assumed to be the most important factor for rapid transfer of water from soil to seed; moreover, in some species 85% or more of the water absorbed by seeds can be directly attributed to vapour (Wuest, 2007). Wetting seeds by solutions of increased osmolality – as a rule high NaCl concentration or PEG solutions – may have a preconditioning effect. Our data show that there was no priming effect of PEG treatment on *Z. lotus* seeds. By experimental transfer to deionized water after 20 days of water stress simulated by PEG-6000 solutions, seeds of *Z. lotus* are able to initiate their germination. Seeds that do not germinate in the presence of water stress constitute a persistent seed bank helping the species in spreading germination over the year.

Burial depth is an important factor regulating seed germination and seedling emergence (Ren et al., 2002). Large losses occur between seed dispersal and seedling emergence because of various reasons. Most plant germination studies have been concerned with the effects of specific environment factors on seed germination and seedling establishment (Zhang, 2001). When buried too deep in the soil, the seeds can either be prevented from germinating by the higher soil moisture, lower temperatures, poor gas exchange, and higher $CO_2$ levels around them (Gutterman, 1993; Keeley and Fotheringham, 1997), or germinate but fail to emerge due to the exhaustion of seedling reserves (Bowers, 1996). In the present experiment, the seeds of *Z. lotus* germinated at all burial depths (1–10 cm), but as burial depth increased seedling emergence decreased, with a percentage over 10% even at a depth of 4 cm. When sand burial depth was 6 cm, a proportion of seeds had germinated but the seedlings failed to emerge above the surface (Fig. 3). For *Z. mauritiana*, seeds should be sown at a depth of 2 cm at 30 × 30 cm spacing in the seedbed any time from spring through the monsoon period (Pareek, 2001); however, sowing seeds at depths more than 3 cm causes poor germination (Azam-Ali et al., 2006).

According to Bond et al. (1999) and Tobe et al. (2007) larger seeds, which have more seed reserves than smaller ones, had the advantage of giving rise to taller seedlings that could emerge from deeper sand. Bond et al. (1999) reported that maximum seedling emergence depth is proportional to the cube root of seed weight. For *Z. lotus* (mean seed weight 33 mg) many deeply buried seeds germinated, but seedling elongation was inhibited (Fig. 3). Our results are similar to those obtained by Tobe et al. (2007) on two deciduous shrubs, *Caragana korshinskii* and *Atraphaxis bracteata* (mean seed weight 65 and 5.7 mg, respectively), that grow on desert sand dunes in China, showing that the large difference in seed weight had no conspicuous effect on the maximum seedling emergence depth. These authors concluded that larger seeds do not always improve seedling emergence. This was in agreement with findings by Pareek (2001) and Azam-Ali et al. (2006) on *Z. mauritiana* (mean seed weight 46.9 mg, Grice, 1996) showing that sowing seeds at depths range from 2 to 3 are preferred and when buried deeply seedling emergence was inhibited. It appears that difference in seed weight between *Z. lotus* and *Z. mauritiana* did not affect the burial depth at which seeds should be sown. Inhibition of seedling elongation can be explained by the exhaustion of seedling reserves and/or may be attributable to stronger hardening of sand with higher water content (Hornbaker et al., 1997). The first day of emergence of the seedlings is strongly related to the temperature of germination and the burial depth. For example, the best emergence of *Z. obtusifolia* var. *obtusifolia* in the field occurred when average soil temperatures ranged from 22.4 to 27.1 °C (Speer and Wright, 1981). However, seeds of *Prosopis glandulosa* buried at depths of 0.5–1.5 cm germinated to 80% at soil temperatures of 27 °C. Maximum seedling emergence under natural temperature regimes occurred after maximum daily temperatures in the surface 2.5 cm of soil were about 25 °C for 4 days (Scifres and Brock, 1972).

From the present study, it can be concluded that seeds of *Z. lotus* have a mechanical dormancy and germinated better at high temperatures and the most suitable temperature found is 35 °C. Germination percentage decreased with increasing moisture stress. It appears that in dunes offering a soil habitat changes there are risks for the seeds to be buried deeply and therefore no emergence. Thus, an intermediate depth of 2 cm is recommended to avoid the risk of seed at low depth drying up before germination and to avoid inhibition of seedling elongation when seeds are placed too deep in the soil. Under natural conditions, seed germination is more complicated and influenced by many factors such as salinity, drought, light and temperature. Future studies would focus on the interactive effects of these factors to better understand the ecophysiological strategies of plants for survival under natural environmental conditions.

## Acknowledgement

We are thankful to Professor Matthew Daws and anonymous referees for their critical reading and revision of the manuscript.

## References

Alves, E.U., Braga Junior, J.M., Bruno, R.L.A., Oliveira, A.P., Cardoso, E.A., Alves, A.U., Alves, A.U., Silva, K.B., 2008. Métodos para quebra de dormência de unidades de dispersão de *Zizyphus joazeiro* Mart (Rhamnaceae). Revista Árvore 32, 407–415.

Aráoz, S.D., Del Longo, O.T., Karlin, U., 2004. Germinación de semillas de *Zizyphus mistol* Grisebach II. Respuestas a diferentes temperaturas y luz. Multequina 13, 41–46.

Azam-Ali, S., Bonkoungou, E., Bowe, C., de Kock, C., Godara, A., Williams, J.T., 2006. Ber. International Centre for Underutilised Crops, Southampton, UK.

Baskin, C.C., Baskin, J.M., 1998. Seeds. Ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego.

Baskin, J.M., Baskin, C.C., 2004. A classification for seed dormancy. Seed Science Research 14, 1–16.

Bewley, J.D., Black, M., 1994. Seeds. Physiology of Development and Germination, 2nd ed. Plenum Press, New York.

Bhansali, A.K., 1975. Monographic Study of the family Rhamnaceae of India. PhD Thesis, University of Jodhpur, India.

Bond, W.J., Honig, M., Maze, K.E., 1999. Seed size and seedling emergence: an allometric relationship and some ecological implications. Oecologia 120, 132–136.

Borgi, W., Chouchane, N., 2009. Anti-spasmodic effects of *Zizyphus lotus* (L.) Desf. extracts on isolated rat duodenum. Journal of Ethnopharmacology 126, 571–573.

Borgi, W., Recio, M.-C., Rios, J.L., Chouchane, N., 2008. Anti-inflammatory and analgesic activities of flavonoid and saponin fractions from *Zizyphus lotus* (L.) Lam. South African Journal of Botany 74, 320–324.

Bowers, J.E., 1996. Seedling emergence on Sonoran Desert dunes. Journal of Arid Environments 33, 63–72.

Danthu, P., Gaye, A., Roussel, J., Sarr, A., 1993. Quelques aspects de la germination des semences de *Ziziphus mauritiana*, In: Somé, L.M., De Kam, M. (Ed.), Les problèmes de semences forestières, notamment en Afrique = [Tree seed problems, with special reference to Africa]. Leiden, Backhuys, pp.192–197. Symposium du groupe de travail IUFRO 'problèmes des semences', 23–27 November 1992, Ouagadougou, Burkina Faso.

Daws, M.I., Ballard, C., Mullins, C.E., Garwood, N.C., Murray, B., Pearson, T.R.H., Burslem, D.F.R.P., 2007. Allometric relationships between seed mass and seedling characteristics reveal trade-offs for neotropical gap-dependent species. Oecologia 154, 445–454.

Daws, M.I., Crabtree, L.M., Dalling, J.W., Mullins, C.E., Burslem, D.F.R.P., 2008. Germination responses to water potential in neotropical pioneers suggest larger seeded species take more risks. Annals of Botany 102, 945–951.

Del Longo, O.T., Aráoz, S.D., 2009. Criteria to determine the duration of the germination test for seeds of 'mistol' (*Ziziphus mistol* Grisebach). Seed Science and Technology 37, 507–511.

Evans, C., Etherington, J.R., 1990. The effect of soil water potential on seed germination of some British plants. New Phytologist 115, 539–548.

Everitt, J.H., 1984. Germination of Texas persimmon seed. Journal of Range Management 37, 189–192.

Finch-Savage, W.E., Leubner-Metzger, G., 2006. Seed dormancy and the control of germination. New Phytologist 171, 501–523.

Gorai, M., Tlig, T., Neffati, M., 2009. Influence of water stress on seed germination characteristics in invasive *Diplotaxis harra* (Forssk.) Boiss (Brassicaceae) in arid zone of Tunisia. Journal of Phytology 1, 249–254.

Grice, A.C., 1996. Seed production, dispersal and germination in *Crytostegia grandiflora* and *Ziziphus mauritiana*, two invasive shrubs in tropical woodlands of northern Australia. Australian Journal of Ecology 21, 324–331.

Griffiths, M.E., Lawes, M.J., 2006. Scarification and maternal plant effects on seedling emergence in *Ziziphus mucronata* (Rhamnaceae). African Journal of Ecology 44, 273–276.

Gutterman, Y., 1993. Seed Germination in Desert Plants. Adaptations of Desert Organisms. Springer-Verlag, Berlin.

Hassen, A., Rethman, N.F.G., Van Niekerk, W.A., 2005. Effect of different seed treatment options on dormancy breaking, germination and emergence of *Ziziphus mucronata* (buffalo thorn) seed. Tropical Grasslands 39, 124–128.

Hornbaker, D.J., Albert, R., Albert, I., Barabási, A.L., Schiffer, P., 1997. What keeps sandcastles standing? Nature 387, 765.

Huang, Z., Gutterman, Y., 1998. *Artemisia monosperma* achene germination in sand: effects of sand depth, water/sand content, cyanobacterial sand crust and temperature. Journal of Arid Environments 38, 27–43.

Hussain, F., Shaukat, S., Ilahi, I., Qureshi, M.Z., 1993. Germination promotion of *Ziziphus nummularia*. Hamdard 36, 46–55.

Jafri, S.M.H., 1977. Rhamnaceae. In: Jafri, S.M.H., EL-Gadi, A. (Eds.), Flora of Libya, Al-Faateh University, Department of Botany, Tripoli, vol. 30, pp. 1–13.

Jordan, C.L., Haferkamp, M.R., 1989. Temperature responses and calculated heat units for germination of several range grasses and shrubs. Journal of Range Management 41, 42–45.

Keeley, J.E., Fotheringham, C.J., 1997. Trace gas emission and smoke-induced seed germination. Science 276, 1248–1250.

Laamouri, A., Zine El Abidine, A., 2000. Multiplication des jujubiers en Tunisie. Annales de la Recherche Forestière au Maroc 33, 37–49.

Le Houérou, H.N., 1959. Recherches écologiques et floristiques sur la végétation de la Tunisie méridionale. Institut des Recherches Sahariennes, Alger.

Liu, M.J., Cheng, J.R., 1994. A taxonomic study on Chinese Jujube and Wild Jujube. Journal of Hebei Agricultural University 17, 1–10.

Michel, B.E., Kaufmann, M.R., 1973. The osmotic potential of polyethylene glycol 6000. Plant Physiology 51, 914–916.

Olmez, Z., Temel, F., Gokturk, A., Yahyaoglu, Z., 2007. Effect of cold stratification treatments on germination of drought tolerant shrubs seeds. Journal of Environmental Biology 28, 447–453.

Pareek, O.P., 2001. Fruits for the Future 2: Ber. International Centre for Underutilised Crops, University of Southampton, Southampton, UK.

PNUD/FAO, 1979. Recherche et développement des parcours du centre-Sud tunisien. Synthèse des travaux réalisés au cours du projet et recommandations. Rapport technique FAO, Rome.

Prins, H., Maghembe, J.A., 1994. Germination studies on seed of fruit trees indigenous to Malawi. Forest Ecology and Management 64, 111–125.

Ren, J., Tao, L., Liu, X.-M., 2002. Effect of sand burial depth on seed germination and seedling emergence of *Calligonum* L. species. Journal of Arid Environments 51, 603–611.

Saied, A., Gebauer, J., Buerkert, A., 2008. Effects of different scarification methods on germination of *Ziziphus spina-christi* seeds. Seed Science and Technology 36, 201–205.

Scifres, C.J., Brock, J.H., 1969. Moisture temperature interrelations in germination and early seedling development of mesquite. Journal of Range Management 22, 334–337.

Scifres, C.J., Brock, J.H., 1972. Emergence of honey mesquite seedlings relative to planting depth and soil temperature. Journal of Range Management 25, 217–219.

Song, J., Feng, G., Tian, C., Zhang, F., 2005. Strategies for adaptation of *Suaeda physophora, Haloxylon ammodendron* and *Haloxylon persicum* to a saline environment during seed germination stage. Annals of Botany 96, 399–405.

Speer, E.R., Wright, H.A., 1981. Germination requirements of lotebush (*Ziziphus obtusifolia* var. obtusifolia). Journal of Range Management 34, 365–368.

Srimathi, P., Sasthri, G., Venkatasalam, E.P., Malarkodi, K., 2002. Efficacy of density grading in ber stones of cv. Umran (*Zizyphus mauritiana* Lamk.). Progressive Horticulture 34, 179–182.

Tengberg, A., Chen, D., 1998. A comparative analysis of nebkhas in central Tunisia and northern Burkina Faso. Geomorphology 22, 181–192.

Tobe, K., Zhang, L., Omasa, K., 2006. Seed germination and seedling emergence of three *Artemisia* species (Asteraceae) inhabiting desert sand dunes in China. Seed Science Research 16, 61–69.

Tobe, K., Zhang, L., Omasa, K., 2007. Seed size effects on seedling emergence of desert psammophytes in China. Arid Land Research and Management 21, 181–192.

Varela, O., Bucher, E.H., 2006. Passage time, viability, and germination of seeds ingested by foxes. Journal of Arid Environments 67, 566–578.

Wuest, S.B., 2007. Vapour is the principal source of water imbibed by seeds in unsaturated soils. Seed Science Research 17, 3–9.

Zhang, Z.B., 2001. Effect of burial and environmental factors on seedling recruitment of *Quercus liaotungensis* Koidz. Acta Ecologica Sinica 21, 374–384.

Zhang, J., Maun, M.A., 1990. Effects of sand burial on seed germination, seedling emergence, survival, and growth of *Agropyron psammophilum*. Canadian Journal of Botany 68, 304–310.

Zhang, S.-Y., Wang, L.-X., 1995. Fruit consumption and seed dispersal of *Ziziphus cinnamomum* (Rhamnaceae) by two sympatric primates (Cebiis apella and Ateles paniscus) in French Guiana. Biotropica 27, 397–401.

Edited by MI Daws